Available online at www.sciencedirect.com
ScienceDirect
Chinese Chemical Letters 22 (2011) 256–259
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

Design, synthesis and biological evaluation of 1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxylic amide derivatives as potential estrogen receptor antagonists

Rui Sun [a], Jing Song [a], Si Jie Liu [a], Hui Zhao [a], Chun Li Yan [a], Ai Jun Zhang [a], Diwa Koirala [b], Da Wei Li [b], Chun Hu [a,*]

[a] *Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, China; Department of Organic Chemistry, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China*
[b] *School of Pharmacy, Shanghai Jiao Tong University, Shanghai 300193, China*

Received 18 June 2010
Available online 24 December 2010

Abstract

The estrogen receptor is a target for therapeutic agents for hormone replacement in menopausal women, osteoporosis, reproductive cancers such as breast cancer, uterine cancer and prostate cancer. 1,4-Dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxylic amide derivatives were designed, synthesized and biological evaluated as potential estrogen receptor antagonists.

Keywords: Estrogen receptor antagonists; Synthesis; Biological activity; Thiophene; Pyrazole; Thiopyran; Piperazine

The estrogen receptor (ER) is a ligand inducible nuclear receptor which play an important role in the reproductive, cardiovascular and central nervous systems and bone tissue. Particularly, ER is a target for therapeutic agents for hormone replacement in menopausal women, osteoporosis, reproductive cancers such as breast cancer, uterine cancer and prostate cancer. The estrogen receptor is found in two subtypes: alpha estrogen receptor (ERα) and beta estrogen receptor (ERβ) [1,2]. The predominant ER in the female reproductive tract and mammary glands is ERα, whereas ERβ is the primary ER in vascular endothelial cells, bone and male prostate tissues [3]. The currently approved antagonists for the estrogen receptor, tamoxifen and raloxifene, are used for the treatment of hormone-dependent breast cancer and osteoporosis, respectively. However, both these agents have been linked to increased risks of thromboembolism, and tamoxifen has been proved to increase the risk of endometrial cancer. Hence, there is a strong need for additional diversity and new chemical scaffolds to allow for exploration of improved tissue selectivity and fewer side effects.

In order to discover some new estrogen receptor antagonists with novel scaffolds, based on the principles of bioisosterism, combination of the active substructures of tamoxifen and raloxifene, and structural optimization, a

* Corresponding author.
E-mail address: chunhu@syphu.edu.cn (C. Hu).

1001-8417/$ – see front matter
doi:10.1016/j.cclet.2010.10.029

novel series of 1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxylic amide derivatives were designed with molecular docking program and synthesized.

For this study, the model was obtained from 3D structural information, of which those protein–ligand complexes are available as PDB files. Based on the obtained hits, 1-(1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carbonyl)-4-(4-trifluoromethoxyphenyl)piperazine (**6d**) was docked into ERα (PDB code 3ERT), showed in Fig. 1 (left). The missed atoms were completed by SPDB Viewer (GSK SPDB Viewer, Version3.7, Swiss). The compound was surrounded by several hydrophobic amino acids, in particular Met343, Leu346 and Thr347, lining the so-called beta face of the ligand-binding domain pocket, Ala350, Asp351, delimiting the alpha one; at the same time, Phe404 defined the bottom wall, while Leu525 formed the side border. The hydrogen-bonding interaction was detected only between **6d** and Arg394 with the hydrogen bond distance of 2.95 Å.

1-(1,4-Dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carbonyl)-4-(4-trifluoromethoxyphenyl)piperazine (**6d**) was docked into the active sites of ERβ (PDB code 1QKM) also. The compound was surrounded by several hydrophobic amino acids, such as Leu298, Met336, Phe356, Ile376 and His475. From Fig. 1 (right) it can be seen that the distance of hydrogen-bond between donor and acceptor is 3.12 Å.

In general, the target compounds (**6a–6e**) were obtained in satisfactory yields, and the synthetic pathways are described in Scheme 1. 3-(Thiophen-2-ylthio)propanoic acid (**1**) were synthesized from 2-mercaptothiophene as the first step. Subsequently, the key intermediate ketone **2** has been prepared according to a literature described by Cagniant and Cagniant [4]. Reaction of **2** with dimethyl oxalate provided **3**. And conversion of **3** to **4** was accomplished by using hydrazine hydrate in acetic acid. Subsequent treatment of the intermediate **4** with sodium hydroxide and then hydrogen chloride gave **5** in nearly quantitative yield. The target compounds **6** could be obtained in the presence of 1-arylpiperazine. All of the target compounds were characterized by NMR and MS [5].

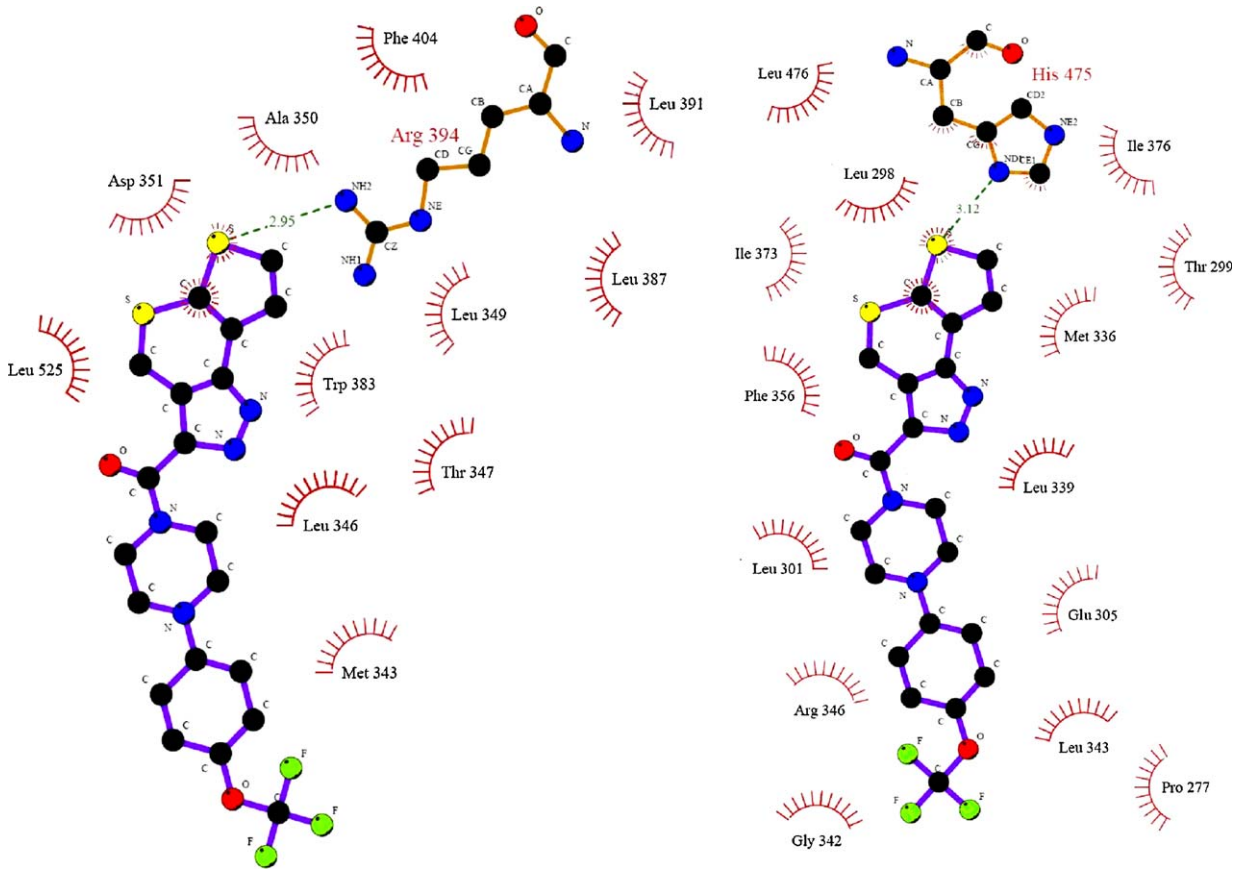
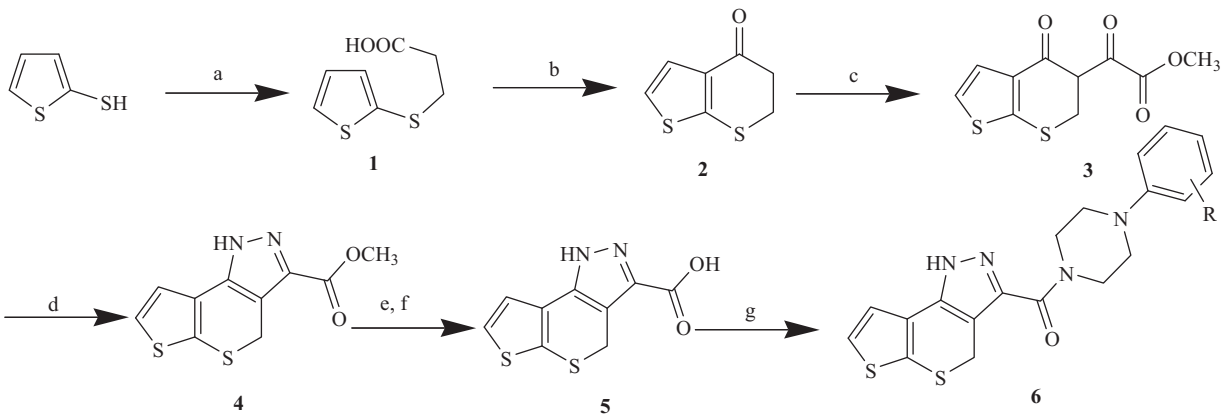


Fig. 1. Docking model of 1-(1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carbonyl)-4-(4-trifluoromethoxyphenyl)piperazine (**6d**) in the active site of human ERα (left) and ERβ (right).



6a: R=H, **6b:** R=2-CH_3,4-CH_3, **6c:** R=3-OCF_3, **6d:** R=4-OCF_3, **6e:** R=4-OCH_3

Scheme 1. The synthetic route of 1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxylic amide derivatives. Condition and reagent: (a) acrylic acid, THF, Et_3N, reflux, 12 h, recrystallized from petroleum ether, yield 80.0%; (b) oxalyl chloride, $SnCl_4$, CH_2Cl_2, r.t., 0.5 h, recrystallized from petroleum ether, yield 76.2%; (c) dimethyl oxalate, sodium methoxide, toluene r.t., 24 h, yield 59.0%; (d) 80% hydrazine hydrate, HOAc, reflux, 12 h, yield 63.2%; (e) NaOH, H_2O, reflux, 2 h; (f) HCl, yield 96.6%; (g) 1-arylpiperazine, EDC, HOBT, CH_2Cl_2, r.t., 24 h, column chromatography on silica with chloroform and methanol [*V*(chloroform):*V*(methanol) = 15:1] as the eluent.

Table 1
The antitumor activities against MCF-7 human mammary tumor cell line.

No.	Inhibition rate (%)	IC_{50} (μmol/L)
6a	22.94	
6b	16.56	
6c	26.30	
6d	71.09	90.63
6e	88.86	72.55
Tamoxifen	100	55.89

The target compounds **6a**–**6e** were initially assessed for their antiproliferative action using the ER expressing (ER dependent) MCF-7 human mammary tumor cell line by MTT assay with tamoxifen as the positive control. The antitumor activities are summarized in Table 1.

Among all the target compounds, the inhibition rate of compound **6d** and **6e** are better than those of the others, while the compound **6a** with no substituent on the benzene ring and **6b** and **6c** with substituent on the C2, C3 position of benzene ring led to a huge decrease in activity which indicates that substituent in the C4 position of benzene ring plays an important role.

In summary, a novel series of 1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxylic amide derivatives are developed as a new class of estrogen receptor antagonists with novel scaffolds. Further efforts aiming at developing potential estrogen receptor antagonists based on modification of compounds **6a**–**6e** would be continued in our laboratory.

Acknowledgment

This work was partially supported by the National Natural Science Foundation of China (NSFC) (No. 20474053).

References

[1] G. Kuiper, E. Enmark, M. Pelto-Huikko, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 5925.
[2] S. Mosselman, J. Polman, R. Dijkema, FEBS Lett. 392 (1996) 49.

[3] K. Dechering, C. Boersma, S. Mosselman, Curr. Med. Chem. 7 (2000) 561.
[4] P. Cagniant, D. Cagniant, Bull. Soc. Chim. Fr. 7 (1966) 2172.
[5] Data for the new compounds. Compd. **6a:** white crystal, yield 36.8%. mp: 235–237 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.24–3.27 (t, 4H), 4.02 (t, 4H), 4.26 (s, 2H), 6.89–6.97 (m, 3H), 7.19–7.20 (m, 1H), 7.26–7.32 (m, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.9, 150.9, 142.0, 136.6, 130.4, 129.1, 126.5, 124.3, 122.7, 119.4, 115.9, 110.1, 49.1, 48.5, 46.3, 41.7, 25.6; ESI-MS: *m/z* 383.1[M+H]$^{+}$, 405.1 [M+Na]$^{+}$. Compd. **6b**: white crystal, yield 57.8%. mp: 228–230 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 3H), 2.17 (s, 3H), 3.12 (s, 4H), 3.77 (s, 2H), 4.10 (s, 2H), 4.28 (s, 2H), 6.69 (t, 1H), 6.80 (s, 1H), 6.98 (d, 1H, *J* = 8.4 Hz), 7.38 (d, 1H, *J* = 2.4 Hz), 7.53 (d, 1H, *J* = 2.7); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.8, 149.2, 142.0, 136.6, 130.4, 130.0, 127.2, 126.5, 124.3, 122.7, 117.8, 113.6, 110.0, 49.7, 49.1, 46.4, 41.8, 25.6, 19.9, 18.5; ESI-MS: *m/z* 411.2[M+H]$^{+}$, 433.1[M+Na]$^{+}$. Compd. **6c**: white crystal, yield 49.0%. mp: 196–198 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.29 (s, 4H), 3.77 (s, 2H), 4.13 (s, 2H), 4.29 (s, 2H), 6.73 (d, 1H, *J* = 7.8 Hz), 6.90 (s, 1H), 6.98–7.00 (m, 1H), 7.32 (t, 1H), 7.38 (d, 1H, *J* = 5.4 Hz), 7.54 (d, 1H, *J* = 5.4 Hz), 13.65 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.9, 152.3, 149.6, 141.9, 136.6, 130.5, 126.5, 124.3, 122.8, 122.0, 119.4, 114.1, 110.5, 107.8, 48.3, 47.7, 46.1, 41.6, 25.6; ESI-MS: *m/z* 467.2[M+H]$^{+}$, 489.1[M+Na]$^{+}$. Compd. **6d**: white crystal, yield 59.3%. mp: 218–220 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.24 (s, 4H), 3.79 (s, 2H), 4.15 (s, 2H), 4.29 (s, 2H), 7.03 (d, 2H, *J* = 9.2 Hz), 7.20 (d, 2H, *J* = 8.6 Hz) 7.21–7.23 (m, 2H), 7.38 (d, 1H, *J* = 5.3 Hz), 7.53 (d, 1H, *J* = 5.3 Hz); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.9, 150.0, 142.0, 140.9, 136.7, 130.4, 126.5, 124.3, 122.8, 121.9, 121.2, 119.5, 116.7, 110.1, 48.9, 48.3, 46.2, 41.6, 25.6; ESI-MS: *m/z* 489.1[M+Na]$^{+}$. Compd. **6e**: white crystal, yield 67.4%. mp: 175–177 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.06 (s, 4H, CH_2), 3.69 (s, 3H), 3.78 (s, 2H), 4.15 (s, 2H), 4.28 (s, 2H), 6.82 (d, 2H, *J* = 9.0 Hz), 6.93 (d, 2H, *J* = 9.0 Hz), 7.37 (d, 1H, *J* = 5.2 Hz), 7.52 (d, 1H, *J* = 5.2 Hz), 13.62 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 161.8, 152.6, 145.2, 142.0, 136.6, 130.4, 126.5, 124.3, 122.8, 118.1, 115.0, 110.0, 63.2, 50.6, 50.0, 46.5, 41.9, 25.5; ESI-MS: *m/z* 435.1 [M+Na]$^{+}$.